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region within the glutathione S-transferase (GST) Pi gene and/or its regulatory flanking sequences, wherein said assay comprises the steps of:

- (i) isolating DNA from said subject,
- (ii) carrying out amplification of said isolated DNA so as to amplify a target region of the GST-Pi gene and/or its regulatory flanking sequences which includes a site or sites at which abnormal cytosine methylation characteristic of the cancer occurs, the amplification being selective in that it only amplifies the target region if the said site or sites at which abnormal cytosine methylation occurs is/are methylated, and
- (iii) detecting the presence of amplified DNA, wherein the detection of amplified DNA is indicative of methylation, and thereby indicative of said cancer,

wherein the amplifying step (ii) is used to amplify a target region within the region of the GST-Pi gene and/or its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +55 represented by nucleotides 1-98 of SEQ ID NOS:52-54.

Claim 4. (Amended) An assay according to Claim 2, wherein said amplification step comprises PCR amplification utilizing a reverse primer including guanine at at least one site whereby, upon the reverse primer annealing to the treated DNA, said guanine will either form a base pair with a methylated cytosine (or another nucleotide to which the methylated cytosine has been

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converted through said treatment) if present, or will form a mismatch with uracil (or another nucleotide to which unmethylated cytosine has been converted through said treatment).

Claim 5. (Amended) An assay according to claim 4, wherein said PCR amplification utilizes a forward primer including cytosine at at least one site(s) corresponding to cytosine nucleotides that are abnormally methylated in the DNA of a subject with the cancer being assayed.

Claim 7. (Amended) An assay according to claim 6, wherein the primers are selected so as to anneal to a sequence within the target region that includes two to four cytosine nucleotides that are abnormally methylated in the isolated DNA of a subject with the cancer being assayed.

Claim 11. (Amended) An assay according to claim 10, wherein said PCR amplification utilizes a forward primer including cytosine at at least one site(s) corresponding to cytosine nucleotides that are abnormally methylated in the isolated DNA of a subject with the cancer being assayed.

Claim 13. (Amended) An assay according to claim 12, wherein the primers are selected so as to anneal to a sequence within the target region that includes two to four cytosine nucleotides that are abnormally methylated in the DNA of a subject with cancer being assayed.

Claim 14. (Twice Amended) An assay according to Claim 1, wherein said DNA is isolated from cells from tissue, blood, blood serum, blood plasma, semen, urine, lymph, or bone marrow.

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Claim 16. (Amended) An assay according to claim 1, wherein the cancer to be assayed is selected from prostate cancer, breast cancer, cervical cancer and liver cancer.

Claim 17. (Amended) An assay according to claim 16, wherein the cancer to be assayed is prostate cancer.

Claim 25. (Amended) An assay according to claim 17, wherein the amplification step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites +1 to +53.

Claim 26. (Amended) An assay according to claim 17, wherein the amplification involves PCR amplification using primer pairs consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers

CGCGAGGTTTCGTTGGAGTTCTCGTC (SEQ ID NO: 1)
CGTTATTAGTGAGTACGCGCGGTT (SEQ ID NO: 2)
YGGTTTTAGGAAATTTTTTCGC (SEQ ID NO: 3)
YGGYGYGTTAGTTYGTTGYGTATATTC (SEQ ID NO: 4)
GGGAATTTTTTCGCGATGTTYGCGC (SEQ ID NO: 5)
TTTTAGGGGTTYGAGCGTT (SEQ ID NO: 6)
GGTAGGTTGYGTTATCGC (SEQ ID NO: 7)

Reverse Primers

TCCCCTCCCTCCCCGAAACGCTCCG (SEQ ID NO: 8)
GAAACGCTCCGAACCCCTAAAAACCGCTAACG (SEQ ID NO: 9)
CRCCCTAAAATCCCCRAAACRCCGCG (SEQ ID NO: 10)
ACCCCRACRACCRCTACACCCRAACGTCG (SEQ ID NO: 11)
CTCTTCTAAAAATCCRCRAACTCCGCG (SEQ ID NO: 12)
AAACRCCCTAAAATCCCCGAAATCGCCG (SEQ ID NO: 13)

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AACTCCCRCRGACCCCAACCCGACGACCG (SEQ ID NO: 14)

AAAAATTCTRAATCTCTCCGAATAACG (SEQ ID NO: 15)

AAAAACCRAAATAAAAACCACACGACG (SEQ ID NO: 16),

wherein Y is C, T or a mixture thereof, and R is A, G or a mixture thereof.

Claim 27. (Amended) An assay according to claim 17, wherein the amplification step involves PCR amplification using primer pairs consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers

CGCGAGGTTTCGTTGGAGTTTCGTCGTC (SEQ ID NO: 1)

CGTTATTAGTGAGTACGCGCGGTTC (SEQ ID NO: 2)

Reverse Primers

TCCCACATCCCTCCCCGAAACGCTCCG (SEQ ID NO: 8)

GAAACGCTCCGAACCCCTAAAAACCGCTAACG (SEQ ID NO: 9).

Claim 28. (Amended) An assay according to claim 17, wherein the amplification step involves PCR amplification using primer pairs consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers

YGGTTTTAGGGAATTTTTTTCGC (SEQ ID NO: 3)

YGGGYGYGTTAGTTYGTTGYGTATATTTC (SEQ ID NO: 4)

GGGAATTTTTTCGCGATGTTYGGCGC (SEQ ID NO: 5)

Reverse Primers

CRCCCTAAATCCCCRAAACRCCGCG (SEQ ID NO: 10)

ACCCCRACRACCRCTACACCCRAACGTCG (SEQ ID NO: 11)

CTCTTCTAAAAATCCRCRAACTCCGCG (SEQ ID NO: 12)

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AAAACRCCCTAAAATCCCCGAAATGCCG (SEQ ID NO: 13)

AACTCCCRCCGACCCCAACCCCGACGACCG (SEQ ID NO: 14),

wherein Y is C, T or a mixture thereof and R is A, G or a mixture thereof.

Claim 29. (Amended) An assay according to claim 17, wherein the amplification step involves PCR amplification using primer pairs consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers

TTTTAGGGGGTTYGGAGCGTTTC (SEQ ID NO: 6)

GGTAGGTTGYGTTATCGC (SEQ ID NO: 7)

Reverse Primers

AAAAATTCTCRAATCTCTCCGAATAAACG (SEQ ID NO: 15)

AAAAACCRAAATAAAACCACACGACG (SEQ ID NO: 16),

wherein Y is C, T or a mixture thereof, and R is A, G or a mixture thereof.

Claim 30. (Amended) An assay according to claim 16, wherein the cancer to be assayed is liver cancer.

Claim 31. (Amended) An assay according to claim 30, wherein the amplification step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to -14.

Claim 34. (Amended) An assay according to claim 30, wherein the amplification step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites +9 to +53.

Claim 35. (Amended) A diagnostic or prognostic assay for a cancer in a subject, said cancer characterised by abnormal

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methylation of cytosine at at least one CpG site in a target region within the glutathione-S-transferase (GST) Pi gene and/or its regulatory flanking sequences, wherein said assay comprises the steps of:

- (i) isolating DNA from said subject, and
- (ii) determining the presence of abnormal methylation of cytosine at a site or sites within the region of the GST-Pi gene and/or its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +55 represented by nucleotides 1-98 of SEQ ID NOs:52-54.

Claim 44. (Twice Amended) An assay according to Claim 35, wherein said DNA is isolated from cells from tissue, blood, blood serum, blood plasma, semen, urine, lymph, or bone marrow.

Claim 46. (Amended) An assay according to claim 35, wherein the cancer to be assayed is selected from prostate cancer, breast cancer, cervical cancer and liver cancer.

Claim 47. (Amended) An assay according to claim 46, wherein the cancer to be assayed is prostate cancer.

Claim 48. (Amended) An assay according to claim 46, wherein the cancer to be assayed is liver cancer.

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Please add the following new claims:

-- Claim 51. A method for the determining abnormal methylation of cytosine at at least one CpG site in a target region within the glutathione-S-transferase (GST) Pi gene and/or its regulatory flanking sequences, wherein said method comprises the steps of:

- (i) contacting a DNA-containing specimen with an agent that modifies unmethylated cytosine in the DNA in said specimen,
- (ii) amplifying the resulting DNA, without prior exhaustive digestion with a restriction enzymes, using primers, wherein the primers are specific for at least one methylated CpG within the target region of the GST-Pi gene and/or its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +55 represented by nucleotides 1-98 of SEQ ID NOS:52-54, and
- (iii) detecting the presence of the at least one methylated CpG site based on the presence or absence of amplification product(s) produced in the amplification step.

Claim 52. The method according to claim 51, wherein the amplification step involves polymerase chain reaction (PCR) amplification.

Claim 53. The method according to claim 51, wherein the modifying agent is bisulfite.

Claim 54. The method according to claim 51, wherein the primers are selected so as to anneal from two to four methylated CpG sites.

Claim 55. The method of claim 51, wherein cytosine is modified to uracil.

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Claim 56. The method according to claim 51, wherein the presence of abnormal methylation of cytosine is indicative or predictive of a cancer characterised by abnormal methylation in the GST-Pi gene and/or its regulatory flanking sequences.

Claim 57. The method according to claim 56, wherein the cancer is selected from the group consisting of prostate cancer, breast cancer, cervical cancer and liver cancer.

Claim 58. The method according to claim 57, wherein the cancer is prostate cancer.

Claim 59. The method according to claim 57, wherein the cancer is liver cancer.

Claim 60. The method according to claim 57, wherein the cancer is breast cancer.

Claim 61. The method according to claim 51, wherein the primers are of 12 to 30 nucleotides in length.

Claim 62. The method according to claim 51, wherein said DNA-containing specimen is cells from tissue, blood, blood serum, blood plasma, semen, urine, lymph or bone marrow.

Claim 63. The method according to claim 51, wherein the primers are specific for at least one CpG site within the GST-Pi gene and/or its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +10.

Claim 64. The method according to claim 51, wherein the primers are specific for at least one CpG site within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to -14.

Claim 65. The method according to claim 51, wherein the primers are specific to at least one CpG site within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to -8.

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Claim 66. The method according to claim 51, wherein the primers are non-specific for at least one of CpG sites -36, -32, -23, -20, -19 and -14.

Claim 67. The method according to claim 51, wherein the primers are reverse and forward primers and where either or both of the reverse or forward primers anneal to a sequence within the target region that includes any or all of CpG sites -36, -32, -23, -20, -19 and -14, then the amplification further utilizes equivalent reverse and/or forward primers including a redundant nucleotide(s) at the position(s) within their sequence(s) corresponding to cytosine or methylated cytosine of one or more of CpG sites -36, -32, -23, -20, -19 and -14.

Claim 68. The method according to claim 51, wherein the primers are designed to minimize the influence of a polymorphic region covering site CpG site -33.

Claim 69. The method according to claim 51, wherein the primers are specific for at least one methylated CpG site within the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites +9 to +53.

Claim 70. The method according to claim 51, wherein the primers are specific for at least one methylated CpG site within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites +1 to +53.

Claim 71. The method according to claim 58, wherein the primers are specific for at least one methylated CpG site located within the region encompassing CpG sites -45 to -8 and/or the region encompassing CpG sites +8 to +53.

Claim 72. The method according to claim 51, wherein the DNA-containing specimen is cells other than from prostate tissue, wherein the primers are not specific for the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -7 to +7.

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Claim 73. The method according to claim 72, wherein the primers are non-specific for the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -13 to +8.

Claim 74. The method according to claim 72, wherein the primers are selective for at least one methylated CpG site within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to -14, -43 to -8, +9 to +53 and +1 to +53.

Claim 75. The method according to claim 59, wherein the primers are selective for at least one methylated CpG site within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to -14 and/or +9 to +53.

Claim 76. The method according to claim 75, wherein in the case of the region being defined by (and inclusive of) CpG sites -43 to -14, the primers are designed so as to minimise the influence of CpG sites -36, -32, -23, -20, -19, -14 and/or a polymorphic region covering site -33. --